ULTRASTRUCTURAL FEATURES OF MACROPHAGE TRANSFORMATION INTO FOAM CELLS in vitro

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The morphological basis of atherosclerotic plaques is formed by foam cells (FC) and connective tissue. Transformation of cells of the vessel wall into FC is observed at sites of its infiltration by apo-B-containing lipoproteins. It is well known, however, that incubation of various cells in medium containing even very high concentrations of native low-density lipoproteins (LDL) does not lead to their subsequent transformation into FC. The use of chemically modified LDL (m-LDL) enabled the process of transformation of smooth-muscle cells (SMC) and macrophages into FC to be reproduced in vitro for the first time. It was shown that the ability of cells to engage in uncontrolled uptake of m-LDL lies at the basis of this phenomenon. It can be postulated that FC formation in an atherosclerotic focus is also the result of the uncontrolled uptake of LDL, modified in the extracellular space of the vessel wall.

Previous investigations showed that the trend of transformations of LDL in the extracellular space of the arterial wall is determined by their aggregation, enzymic modification, and accumulation of lipid peroxides in their composition and complex formation with glycosaminoglycans [2].

The aim of this investigation was to study the cellular mechanisms of macrophage transformation into FC during their incubation with aggregated LDL (a-LDL) and fibroblast-modified LDL (fm-LDL).

EXPERIMENTAL METHOD

Experiments were carried out on cultured human embryonic lung fibroblasts at the 10th passage and on mouse peritoneal macrophages. LDL (1.019-1.055 g/ml) and blood serum proteins not containing LP, with a density of 1.250 g/ml, were isolated by ultracentrifugation [6] from blood of a human blood donor. Lipoproteins (LP) used in the experiments were estimated as protein [7]. To obtain aggregates of LDL a solution of native LDL from human blood plasma, dialyzed against Hanks' solution, was incubated for 3-7 days at 37°C under sterile conditions. A subfraction of aggregates (a-LDL) was then isolated from the total LDL fraction by gel filtration on Sepharose 4B.

To obtain fibroblast-modified LDL, the cells were incubated in Eagle's medium containing 50 μg/ml of native LDL and 5 mg/ml of nonlipoprotein blood serum proteins with a density of more 1.250 g/ml for 2 days. The medium was then poured off, concentrated by means of Amicon on an XM-300 filter, and dialyzed against fresh Eagle's medium. This procedure enabled the LDL to be separated from nonlipoprotein proteins. The fm-LDL thus isolated were used for incubation with macrophages.

Mouse peritoneal macrophages [5] were cultured on plastic supports in 16-mm Multiwells (Falcon Plastics, USA) for 6 days. Eagle's medium containing a-LDL or fm-LDL in a concentration of 50 μg/ml, and nonlipoprotein proteins (5 mg/ml), was used as the incubation medium. The incubation medium was changed for fresh every 2 days. After the end of incubation the cells were washed off five times with 0.15 M phosphate buffer, pH 7.4 [3], and fixed in glutaraldehyde in cacodylate buffer, pH 7.4. The subsequent processing and embedding of the material were carried out by the method described previously [1].

EXPERIMENTAL RESULTS

During incubation of mouse macrophages with fm-LDL or a-LDL for 6 days, FC were observed to be formed, whereas during incubation of macrophages with native LDL no FC were formed. Besides FC formed

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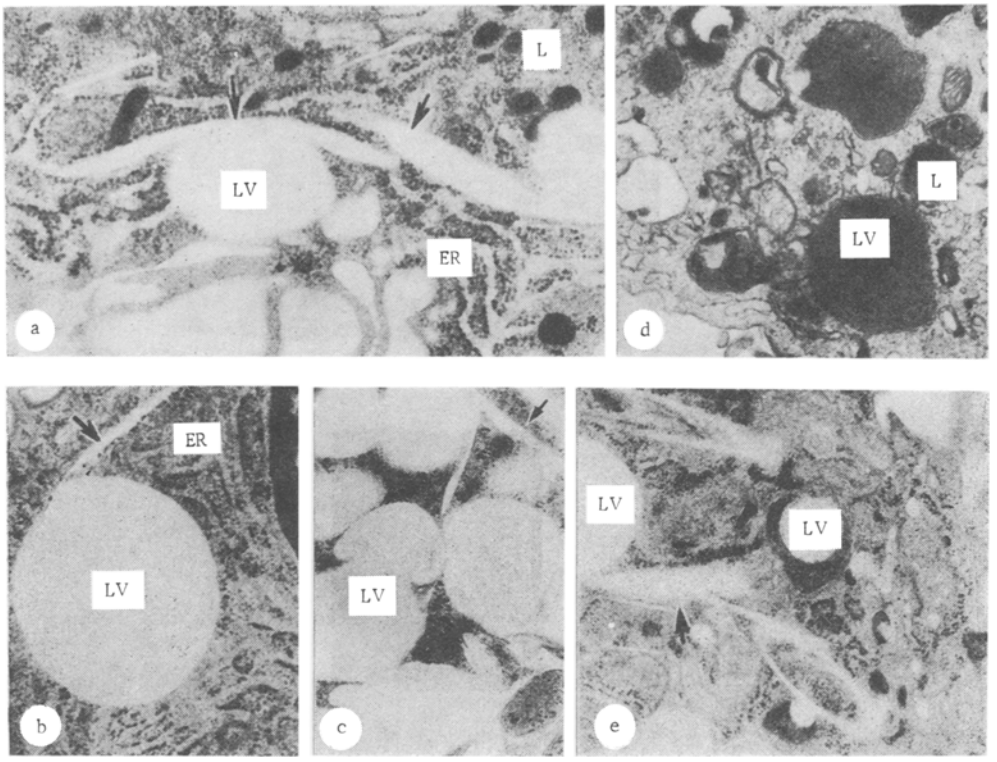

Fig. 1. Ultrastructural features of lipid vacuole formation in macrophages undergoing transformation into foam cells. A) Cisterns of rough endoplasmic reticulum filling with lipids, 66,000 ×; b) formation of a large, membraneless lipid vacuole, 46,000 ×; c) merging of membraneless lipid vacuoles, filling cytoplasm of cell, 26,000 ×; d) formation of large lipid vacuoles surrounded by a membrane, 60,000 ×; e) formation of translucent lipid vacuole (in center), surrounded by membrane, 46,000 ×. LV) Lipid vacuoles, ER) endoplasmic reticulum, L) lysomes. Arrows indicate cisterns of rough endoplasmic reticulum, filled with lipids.

previously, the initial stages of transformation of macrophages into FC could also be seen. Morphological analysis revealed no significant difference in the intensity of FC formation whether fm-LDL or a-LDL were used. The ability of macrophage to engage in uncontrolled uptake of these LP is evidently a triggering mechanism, inducing their transformation into FC.

Since similar changes in the structure and properties of LDL can take place in the vessel wall also, the observations described above can shed some light on the principles governing FC formation in the arterial wall.

Ultrastructural analysis of transformation of macrophages into FC revealed some special features of lipid vacuole formation in the cell cytoplasm not previously described in the literature. Analysis of the mechanisms of this uncontrolled endocytosis of m-LDL [5] demonstrates that the processes of endocytosis of m-LDL, their subsequent degradation in the lysosomes, and re-esterification of the free cholesterol thus liberated, with the aid of the microsomal enzyme acyl-CoA-cholesterol acyltransferase (ACAT), with the formation of membraneless lipid vacuoles, are coordinated.

A consecutive study of the various stages of FC formation in the course of this investigation showed that, before the formation of lipid vacuoles, the hypertrophied cisterns of the endoplasmic reticulum, with a uniform distribution of ribosomes on the membranes (Fig. 1), filled up with esterified cholesterol. Accumulations of lipids were, therefore, varied in shape, repeating the structure of the hypertrophied cisterns, and sometimes merging with one another. Often these concentrations of lipids resembled cholesterol crystals, but the latter are always surrounded by membranes. They are probably phospholipid in nature, like myelin-like structures, and usually are not bound to the endoplasmic reticulum.

As a result of esterification of cholesterol, membraneless lipid vacuoles of low electron density formed in the rough endoplasmic reticulum (Fig. 1b). In the course of this process ribosomes on membranes of the cisterns gradually disappeared, after which the cisterns themselves melted. Large and small membraneless lipid vacuoles joined together and gradually replaced the endoplasmic reticulum (Fig. 1c). These observations suggest the existence of another pathway of lipid vacuole formation in macrophages. In cells incubated with a-LDL and with fm-LDL, besides typical membraneless vacuoles, it was also possible to observe large endocytotic vesicles, more than 100 nm in diameter, surrounded by a membrane, and possessing average electron density (Fig. 1d). Endocytotic vesicles of this kind (we call them "vacuoles surrounded by an LP membrane"), as a rule, do not merge with lysosomes (Fig. 1d). As the lipid vacuoles surrounded by a membrane accumulate in the zone of the endoplasmic reticulum, they become translucent (probably because of esterification of cholesterol), starting from the periphery, and gradually extending into the central part of the vacuole (Fig. 1d, e). In the stage of complete translucency, these lipid vacuoles differ from the membraneless kind only in that they are surrounded by a membrane. The re-esterification of cholesterol probably takes place on account of nonspecific esterase and ACAT, for the rough endoplasmic reticulum, which also surrounds lipid vacuoles, is grossly hypertrophied.

It can be tentatively suggested that the presence of this second pathway of formation of lipid vacuoles in FC is the result either of the existence of different mechanisms of uncontrolled endocytosis of m-LDL in the cell, or of the relative functional insufficiency of the lysosomal apparatus when an excess of m-LDL enters the cell.

Modification of the properties of LDL is thus a factor inducing rapid transformation of macrophages into FC. Under these circumstances the process of catabolism of m-LDL or fm-LDL and the formation of lipid vacuoles take place in two ways: with or without the participation of lysosomes. In the first case membraneless lipid vacuoles are formed, in the second case – vacuoles surrounded by a membrane.

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